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# A Multistep Mechanism of Nucleophilic Substitution of Vitamin $B_1$ in Methanol

JOHN A. ZOLTEWICZ[1] AND THOMAS D. BAUGH

*Department of Chemistry, University of Florida, Gainesville, Florida 32611*

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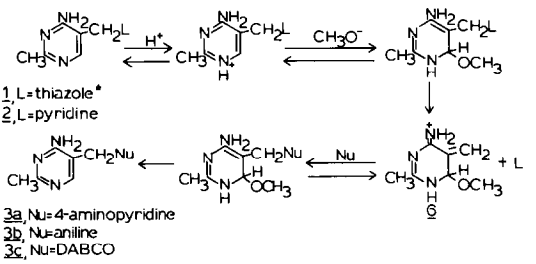
Thiamin (**1**) and an analog (**2**) having a pyridine ring in place of the thiazole portion react with amine nucleophiles in methanol at 71.5°C to give substitution products **3**. The nucleophiles replace the thiazole and pyridine rings. The course of the substitution reactions which were followed by PMR are first order in substrate and zero order in amine. Proposed is a multistep mechanism analogous to that observed for second-order reactions between sulfite ion, for example, and thiamin, its derivatives, and analogs. The conjugate acid of the substrate forms a sigma adduct with methoxide ion. Following fragmentation to a resonance-stabilized cation the amine nucleophile becomes incorporated into this charged intermediate. Loss of methoxide ion generates the aromatic substitution product. Substrate protonation and methoxide ion addition are kinetically equivalent to the addition of solvent, a process that cannot be detected kinetically. Addition of the amine occurs in a fast step. Overall then, the scheme is only first order in substrate as required by the experimental observations. 

## INTRODUCTION

Nucleophilic substitution on thiamin (**1**, vitamin $B_1$) is rarely observed. Of the few examples reported, the best known is the historically and chemically important cleavage by sulfite ion in water (*1*, *2*). More recent extensions include the related sulfite ion catalyzed competition for **1** by various nucleophiles (*3*, *4*). The lack of substitution in water is readily understood because under the alkaline conditions required to generate many powerful nucleophiles the thiazolium ring of the vitamin is rapidly cleaved by the action of hydroxide ion to give a ring-opened amine that is a poor leaving group (*5*). Perhaps the next most significant substitu-




SCHEME 1

[1] To whom correspondence should be addressed.



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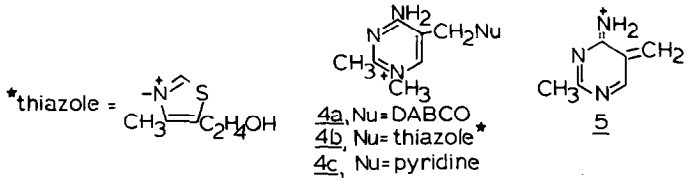
tion reaction of thiamin is its self-quarternization in alcoholic solvents to yield oligomers (*6, 7*).

We wish to report that **1** may be made to undergo nucleophilic substitution in methanol solvent. Under our conditions both thiazolium ring-opening and oligomerization are unimportant. A range of amines successfully brings about substitution. Our kinetic studies reveal the mechanism to be multistep, similar to that we reported for **1** and sulfite ion in water (*2*).

## RESULTS

In heated dry methanol **1** and its analog, **2,** readily undergo nucleophilic substitution. The thiazole ring of **1** and the pyridine ring of **2** were replaced by four nucleophiles: pyridine, 4-aminopyridine, aniline, and diazabicyclo[2.2.2]octane (DABCO). The structures of the pyridine (**2**) and 4-aminopyridine (**3a**) products were confirmed by isolation, the aniline product **3b** by comparison of its NMR spectrum with that of an authentic sample. It was not possible to isolate the product **3c** formed by a reaction between DABCO and **1**. However, a derivative **4a**




was easily synthesized. This compound has a methyl group at position 1 of the pyrimidine ring and is prepared from DABCO and 1′-thiaminium ion **4b** (*8*). The presence of the methoxide ion substitution product, 4-amino-5-methoxymethyl-2-methylpyrimidine (*9*), was not detected.

An earlier study reports that **1** in 95% methanol reacts with pyridine to give the expected substitution product **2** (*6*). We synthesized **2** by this method.

The kinetics of substitution of **1** with the four nucleophiles and of **2** with DABCO in methanol at 71.5°C were obtained using PMR to follow reactions. In most instances, Table 1, the nucleophile was present in about a two- to fourfold excess over that of the substrate. In all cases, however, the concentration–time data are better fitted to a first- rather than to a second-order plot. Moreover, increasing the ratio of amine to substrate had no significant influence on the magnitude of the observed first-order rate constant. For example, with pyridine-$d_5$ as the nucleophile, changing this ratio from 2 : 1 to 10 : 1 only increased the first-order rate constant by 13%, an insignificant amount. This value is similar to the 10% average deviation found for all eight kinetic results on this substrate. Deuterated pyridine was employed so that its signal would not overlap with that of the liberated thiazole and thus would not hamper signal integration.

Another significant feature of the data is that all the first-order rate constants for **1** are independent of the identity of the amine and are the same within experimental error, the average being $1.7 \pm 0.2 \times 10^{-5}$ $s^{-1}$. The average half-life is 11.3 h.

TABLE 1

RATE CONSTANTS AND CONDITIONS FOR THE SUBSTITUTION OF **1** AND **2** BY NUCLEOPHILES IN METHANOL AT 71.5°C

| [Cpd] (M) | Nucleophile | [Nucleophile] (M) | | $10^5 k_\psi$ ($s^{-1}$) |
|---|---|---|---|---|
| **1**, 0.095 | Pyridine-$d_5$ | 1.0 | | 1.88 |
| **1**, 0.19 | | 0.95 | | 1.88 |
| **1**, 0.19 | | 0.38 | | 1.66 |
| **1**, 0.19 | 4-$NH_2$pyridine | 0.95 | | 1.47 |
| **1**, 0.38 | | 1.1 | | 1.85 |
| **1**, 0.47 | | 0.95 | | 1.51 |
| **1**, 0.56 | Aniline | 1.7 | | 1.50 |
| **1**, 0.47 | DABCO[a] | 0.95 | | 1.84 |
| | | | average | 1.70 ± 0.17 |
| **2**, 0.38 | DABCO[a] | 1.1 | | 2.00 |
| **2**, 0.47 | | 0.95 | | 1.85 |
| | | | average | 1.92 ± 0.08 |

[a] Diazabicyclo[2.2.2]octane.

This is so in spite of the wide variation in basicities and expected nucleophilicities of the bases examined, including primary and tertiary amines.

Two kinetic runs were performed with **2**, both involving DABCO. The average rate constant, $1.9 \pm 0.1 \times 10^{-5}\ s^{-1}$, is not significantly different from that for **2**. By comparison, in a reaction with sulfite ion in water **1** is 2.0 times less reactive than **2** at 25°C. (*2*).

Under our conditions, employing buffers, any intramolecular addition of the 4′-amino group to the thiazolium ring of **1** to form fused ring products is insignificant. Such cyclization requires two (*10*) or three (*11*) equivalents of methoxide ion, the nature of the product depending on the stoichiometry.

## DISCUSSION

The fit of the data to first-order plots and the absence of a rate dependence on the concentration and identity of the nucleophile clearly eliminate an $S_N2$ mechanism. Instead, one or more intermediates must be present in the reactions of **1** and **2** with amine nucleophiles in methanol. The simplest and most obvious mechanism is $S_N1$ in which the thiazole or pyridine leaving group departs in the rate-limiting step to give the resonance-stabilized carbocation, **5**. This cationic intermediate then reacts in a subsequent fast step with the nucleophile to give the observed product. Such a mechanism has been provided as speculation for the hydrolysis reaction of the 5-chloro analog of thiamin (4-amino-5-chloromethyl-2-methylpyrimidine) (*12*). Although such a mechanism is kinetically consistent with our results we disfavor it and instead advance an entirely different pathway, one that is related to the common mechanism found for **1**, its *N*-methylated derivative,

and its *N*-methylated analogs. Such substrates react with aqueous sulfite (*2, 13, 15–17*) and hydroxide ions (*14*) by a multistep process. In addition, the *N*-methylated analogs react by a similar pathway with methoxide ion in methanol (*7*).

In the multistep pathway occurring in methanol we suggest that **1** and **2** must first be protonated, probably at their thermodynamically favored N-1 position (Scheme 1). This is then followed by addition to the cation of methoxide ion formed in a solvolysis reaction with the added amine nucleophile. For steric reasons the more accessible 6 position is favored for addition. These two steps are kinetically equivalent to the addition of solvent to the substrate. Moreover, since reaction with both acid and base are involved, the overall process is pH independent, and this is consistent with our observations. Following these two steps the leaving group departs to form resonance-stabilized cation, **6**. Capture of this cation or its conjugate base by a nucleophile and subsequent aromatization gives the observed product in fast steps. Either the addition of methoxide ion to the protonated ring or the loss of the nucleofuge is likely to be the rate-limiting step. Our data do not allow a distinction to be made. Both possibilities are consistent with the observed first-order kinetics as shown by Eq. [1], where $k_\psi$ is the observed pseudo-first-order rate constant, $k$ is the second-order constant for addition of methoxide ion to the pyrimidinium ion, and $K_a$ is the dissociation constant for the conjugate acid of the substrate. Moreover, when the fraction of protonated substrate is low, i.e., when $[H] < K_a$, then $k_\psi$ is a pH-independent constant, Eq. [2], where $K_s$ is the ion product for methanol.

$$k_\psi = \frac{k[H][CH_3O^-]}{[H] + K_a} \quad [1]$$

$$k_\psi = \frac{kK_s}{K_a} \quad [2]$$

If loss of the leaving group is rate-limiting, then another equilibrium constant to express the reversible addition of methoxide ion to the pyrmidinium ion must be added to the equations; $k$ then becomes the first-order rate constant for the fragmentation reaction to yield cation **6**.

The methanolysis reaction differs from the reaction of sulfite ion and **1** or **2**, and also from the reaction of the *N*-methylated analog of **2**, i.e., **4c**, with hydroxide ion. These substitution reactions are clearly second order, first order in substrate, and also first order in sulfite or hydroxide ions. The kinetic dependence on lyate ion in the hydrolysis reactions is experimentally detectable because the substrate need not undergo protonation; the substrate already is activated for nucleophilic attack by being *N*-methylated. Moreover, a wide variety of *N*-methylated thiamin analogs, upon reacting in methanol, also show a kinetic dependence on methoxide ion, i.e., they are second-order overall when they undergo nucleophilic substitution. For example, when **4c** reacts to give the DABCO substitution product, **4a**, in methanol the reaction is first order in both substrate and methoxide ion (*18*).

Strong support for our suggested mechanism comes from comparison of the reactivity of **2** with that of its *N*-methylated derivative, **4c**. The *N*-methylated compound is so reactive that experiments were conducted in methanol at 25°C

instead of the 71.5°C employed in the present studies. Hence, it is necessary to convert the kinetic data to a common temperature. The pseudo-first-order rate constant for **2** may be converted to a second-order constant using Eq. [2] and estimates of its $pK_a$ of 4.8 (*2*)[2] and the ion product of methanol ($pK_s$ = 16.5) (*20*) at 71°C. Assuming a reasonable energy of activation of 20 kcal/mol and reducing the temperature to 25°C should make the value be $10^2$ times smaller, or about $1 \times 10^5$ $M^{-1}$ $s^{-1}$. The measured rate constant for the *N*-methyl compound **4c** is $2.6 \times 10^4$ $M^{-1}$ $s^{-1}$ (*18*), essentially the same as the temperature corrected value for **2**. This agreement shows that the free and quaternized bases react by a common mechanism. The high value for the second-order rate constant is noteworthy. The lower apparent reactivity of the free base is merely a reflection of its lower degree of conversion to the reactive cationic pyrimidine structure under the reaction conditions.

The rate limiting step for the reaction of **4c** with methoxide ion is believed to be the loss of the pyridine ring to give the *N*-methylated equivalent of **6** (*18*). Presumably, the same step also applies to **2.** Therefore, the apparent second-order rate constants for **4c** and **2** include the equilibrium constant for methoxide ion addition and the rate constant for fragmentation.

According to our proposed mechanism (Scheme 1), methoxide ion, which is present in far lower concentrations than the amine,[3] catalyzes the substitution reaction but it does not appear in the major product. These curious observations may be readily understood, however. Methoxide ion rather than the amine serves as the catalyst because it is not merely a better nucleophile than the amine (*21, 22*). It also is a poorer leaving group than the amine (*21, 23*). This latter property is critical to its success as the catalyst. Methoxide ion not only attacks the ring, it also remains bonded to it long enough to allow the leaving thiazole or pyridine to depart. If it did not remain attached to the ring for this length of time, there would be no forward reaction. Amine nucleophiles, by contrast, add to the pyrimidine ring but depart before the group leaves the side chain and so they have no influence on the rate. In other words, the rate constant for amine loss is greater than that for methoxide ion departure.

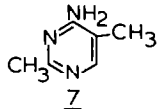
The same possibilities of addition and departure of a nucleophile also pertain to trapping of intermediate **6** at its side chain (Scheme 1). But, in this case, the timing of the loss of the nucleophile from the chain is less critical. Rearomatization is so facile that even groups with better leaving properties will remain bonded to the side chain long enough to allow methoxide to depart from its annular position. Therefore, the more abundant amine nucleophile appears bonded at the methylene position in the major product. Its greater concentration ensures that it will react faster than methoxide ion.

Our proposed mechanism provides an attractive explanation for some heretofore puzzling reactions of **1** in methanol in the presence of aromatic aldehydes and

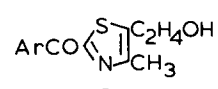
[2] The temperature-dependent correction factor for **2** was estimated using $pK_a$ data for 4-picoline in methanol (*19*).

[3] For example, if the concentration of pyridine is 1 M, then the methoxide concentration is only about $2 \times 10^{-6}$ M.

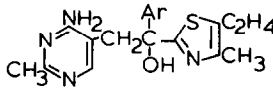
triethylamine. On heating this mixture at reflux for 5 h a redox reaction takes place: 2-amino-2,5-dimethylpyrimidine (**7**) and 2-aroylthiazole (**8**) are isolated along with trace amounts of a carbinol "insertion product," **9**, and a benzoin. Significant observations concerning reactivity are: (i) the precursor to products may be intermediate **10** (G = aryl) produced when the thiazolium ylide formed on deprotonation of **1** captures aldehyde; (ii) analogs not having the pyrimidine ring do not undergo the redox cleavage reaction; (iii) oxythiamin, i.e., the 4′-oxo derivative of **1**, does not react; and (iv) aliphatic aldehydes yield intermediate **10** (G = alkyl) and do not show the cleavage step. Previously, these observations were interpreted in terms of an intramolecular rearrangement, special emphasis being placed on the need to have an amino group bonded to the pyrimidine ring (*24*).



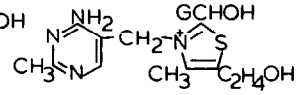
**7**



**8**



**9**



**10**

We suggest an entirely different explanation, one based on the chemistry in Scheme 1. Protonated carbinol intermediate **10** reacts with methoxide ion to give cation **6** and the 2-hydroxyaryl derivative of the thiazole. Then **6** abstracts hydride ion to give the observed reduced pyrimidine, **7**, and the oxidized side chain of the thiazole. The hydride donor may be alcohol **10** and/or the liberated hydroxyaryl thiazole. Since cation **6** is the hydride acceptor it is apparent that analogs lacking the necessary precursor pyrimidine ring will not undergo the redox reaction. The lack of reaction of oxythiamin is consistent with the report that this material is inert in methanol (*6*), in contrast to **1**, which rapidly reacts both in the presence and absence of added nucleophiles (*6*, *7*). Finally, the insertion product arises when hydroxyaryl thiazole undergoes deprotonation at the carbinol carbon to form a nucleophile. This carbon nucleophile then couples with **6**. It is not clear whether the nucleophile is formed from intermediate **10** or from the liberated hydroxyaryl thiazole. Should the former nucleophile be the enamine of **10**, then another **6** would have to be liberated from the thiazole ring according to Scheme 1 to give the observed insertion product following coupling. The absence of a cleavage reaction with aliphatic aldehydes is curious. It may be related to the ease with which the resultant carbinol undergoes cleavage according to the pathway in Scheme 1; reaction times were not reported. We find that **1** has a half-life of 11.3 h at a temperature similar to that of refluxing methanol.

The present conclusions regarding the mechanism of substitution of **1** and **2** have some significance for the reaction of these substrates with thiaminase I. Both compounds undergo nucleophilic substitution in the presence of this enzyme, and the mechanism has been shown to be of the ping–pong type, although the nature of the intermediate has not yet been identified (*12*). Scheme 1 appears attractive, a

nucleophilic site on the enzyme acting in place of methoxide ion to initiate substitution.

## CONCLUSIONS

Both **1** and **2** react with amines in methanol to give products **3** by a multistep process in which methoxide ion, not the amine, acts as a catalyst.

## EXPERIMENTAL PROCEDURES

*Materials.* Thiamin hydrochloride (Sigma) and the hydrochloride salt of **2** (*6*) were vacuum-dried over magnesium perchlorate for 24 h and stored in a vacuum dessicator. Aniline was distilled before use; 1,4-diazabicyclo-[2.2.2]-octane was recrystallized from cyclohexane. 4-Aminopyridine was recrystallized from benzene. Benzyltriethylyammonium perchlorate (BTAP) was prepared from triethylamine and benzyl bromide followed by recrystallization from aqueous sodium perchlorate. Reagent-grade methanol was heated at reflux over magnesium turnings and distilled onto 3-Å molecular sieves that were activated by heating at 300°C for 24 h and stored at 110°C. All glassware was oven dried for 12 h prior to use.

1-[(2-Methyl-4-amino-5-pyrimidinyl)methyl]pyridinium chloride hydrochloride (**2**), mp 256–258°C dec. [lit (*6*) 258°C], was prepared from the hydrochloride of **1** in 95% methanol (*6*). Its NMR spectrum is as follows: $^1$H NMR ($Me_2SO$-$d_6$, $Me_4Si$) $\delta$ 2.56 (2′-$CH_3$), 3.88 ($NH_2$, $H_2O$), 6.01 ($CH_2$), 8.19 ($H_{3,5}$, dd, $J_{2,3} \cong J_{3,4} = 6$ Hz), 8.67 ($H_6'$, s, $H_4$, t, $J = 6$ Hz), 9.23 ($H_2$, $H_6$, d, $J \cong 6$ Hz).

*N*-[(2-Methyl-4-amino-5-pyrimidinyl)methyl]anilinium hydrochloride (**3b**), mp 114–116°C [lit. (*25*) 114–116°C], was prepared using aqueous sulfite ion (*25*). It has the following NMR spectrum: $^1$H NMR ($Me_2SO$-$d_6$, $Me_4Si$) $\delta$ 2.50 (2′-$CH_3$), 3.63 [$NH_2$ or (NH), $H_2O$], 4.17 ($CH_2$), 6.67 ($H_2$, $H_4$, $H_6$), 7.13 ($H_3$, $H_5$), 8.10 ($H_6'$), 8.93 [$NH_2$ or (NH)].

Product **4a** was prepared from **4b** and DABCO (*18*).

*Preparation of* 1-[(4-*amino*-2-*methyl*-5-*pyrimidinyl*)*methyl*]-4-*aminopyridinium chloride hydrochloride*, **3a**. Thiamin chloride hydrochloride (5.00 g, 14.8 mmol), oven-dried at 110°C for 14 h, and 4-aminopyridine (4.19 g, 44.7 mmol), recrystallized from benzene and vacuum-dried over magnesium perchlorate, were placed in a heavy-walled glass ampoule. Methanol (25 ml) was added and the ampoule was flame-sealed. The solution was heated at 100°C for 4 h. The solvent was removed under reduced pressure to give a yellowish semisolid that was taken up in water (10 ml). The basic solution was extracted with ethyl acetate (5 × 10 ml) to remove the liberated thiazole and excess 4-aminopyridine. The aqueous solution was acidified with concentrated hydrochloric acid (10 ml) and the solvent was removed under reduced pressure. The resulting solid was extracted with hot 95% ethanol (3 × 20 ml) to separate the organic compounds from sodium chloride. The solvent was again evaporated under reduced pressure and the remaining solid was

recrystallized from 95% ethanol/acetone. The yield of product was 0.869 g (20%, 3.00 mmol) of colorless needles; mp 278–282°C (dec.): $^1$H NMR ($D_2O$, TSP) δ 2.70 (2′-$CH_3$), 5.47 ($CH_2$), 7.05 ($H_3$, $H_5$, d, $J_{2,3}$ = 7 Hz), 8.15 ($H_2$, $H_6$, d, $J_{2,3}$ = 7 Hz), 8.12 ($H_6'$); $^{13}$C NMR($Me_2SO$-$d_6$, $Me_4Si$) δ 25.3 (2′-$CH_3$), 53.7 ($CH_2$), 107.1 ($C_5'$), 109.5 ($C_3$, $C_5$), 142.4 ($C_2$, $C_6$), 157.2 ($C_6'$), 158.8 ($C_4$), 161.6, 167.7 ($C_2'$, $C_4'$).

*Kinetics.* Samples were prepared by weighing the substrate and nucleophile into an NMR tube. Methanol was added to the tube in a drybox or glove-bag flushed with nitrogen. The solution volume was adjusted to 0.50 ml by comparison with the liquid level in another NMR tube to which 0.50 ml of methanol had been added. Two or three molecular sieve pellets were added to the solution and the tube was then sealed with a flame. The time-zero spectrum was taken and the tube was placed in a water bath maintained at 71.5°C. The progress of the reaction was followed by periodically recording the NMR spectrum at probe temperature. Reactions were followed for at least 1.5 half-lives, with some up to 4 half-lives.

The reaction of **1** with nucleophiles was followed by monitoring the dsappearance of the H-2 signal for the thiazolium ring and the appearance of the H-2 signal of the liberated thiazole (*T*). The extent of (*T*) formation was determined by Eq. [3], where *A* denotes the average area of the NMR signal from at least four integrations:

$$\%(T) = \frac{A \text{ H-2 of } (T)}{A \text{ (H-2 of } \mathbf{(1)} + \text{H-2 of } (T))} \times 100. \qquad [3]$$

The amount in **1** remaining was calculated according to

$$[\mathbf{1}] = [\mathbf{1}]_0 - [\mathbf{1}]_0 \times \%(T) \times 10^{-2}. \qquad [4]$$

The reactions of **2** with nucleophiles were followed by monitoring the disappearance of the H-2,6 and H-4 signals of **2** and the appearance of the H-2,6 signals of the liberated pyridine (*P*). The H-2,6 signal of **2** appears as a multiplet at 9.10 ppm; the multiplet moves upfield to 8.60 ppm as **2** releases (*P*). The H-4 signal of **2**, however, appears as a multiplet at 8.75 ppm and overlaps the H-2,6 signals of (*P*). Due to overlap the area of the H-2,6 protons of (*P*) was calculated by subtracting the area of the H-4 proton of **2** from the total area of the overlapped region. The extent of (*P*) formation was then determined by Eq. [5], where *A* denotes the average integrated area of at least four determinations:

$$\%(P) = \frac{A(\text{H-2,6 of } (P) + \text{H-4 of } (\mathbf{2})) - 0.5A(\text{H-2,6 of } (\mathbf{2}))}{A(\text{H-2,6 of } (P) + \text{H-4 of } (\mathbf{2})) + 0.5A(\text{H-2,6 of } (\mathbf{2}))} \times 100. \qquad [5]$$

The amount of **2** remaining was calculated according to

$$[\mathbf{2}] = [\mathbf{2}]_0 - [\mathbf{2}]_0 \times \%P \times 10^{-2}. \qquad [6]$$

In all cases rate constants, $k_\psi$, were obtained from slopes of plots of ln(%H remaining) vs. time. Concentrations of nucleophiles and substrates reported in Table 1 were corrected for thermal expansion of methanol. Concentrations determined at ambient temperature, assumed to be 25°C, decrease by 5.7% on heating (*26*).

*Mass balance and product verification.* A mass balance for the conversion of **1**

and pyridine-$d_5$ to **2-$d_5$** and the free thiazole was determined by comparing their combined areas to that of the aromatic protons of internal standard 1,3,5-trimethylbenzene, 6.78 ppm. The areas of the H-2 signal of the thiazole ring in **1** and in free thiazole, 9.5–9.8, and 8.8 ppm, respectively, were measured. The total amount of thiazole in reactant and product was compared to the standard for each kinetic point in runs 1 and 2 (Table 1). The mass balance observed in run 1 was 102 ± 5% over 1.7 half-lives. Similar results were found in run 2, 95 ± 7% over 1.4 half-lives. The conversion of **1** to **2-$d_5$** is therefore the major process for the reaction of **1** with pyridine-$d_5$.

The appearance of the methylene signal of product **2-$d_5$**, 6.02 ppm, was compared to the appearance of the H-2 signal of free thiazole periodically during the course of the reaction above. In all cases the amounts of product were equal as determined by area integration. Formation of **2-$d_5$** is therefore correlated with the liberation of thiazole. On a preparative scale **2** was isolated in 60% yield.

A mass balance for a reaction of **2** and DABCO in methanol was investigated. The signals attributed to **2** and to the substitution products were integrated with respect to internal standards, BTAP and sodium 3-trimethylsilylpropionate-2,2,3,3-$d_4$ (TSP). The ratio of **2** to standard was obtained by integration of H-2,6, 9.09 ppm, or the methylene group of **2** and the methyl triplet, 1.27 ppm, or aromatic signal, 7.63 ppm, of BTAP or the methyl signal of TSP, 0 ppm. The reaction was run for 5 h at 100°C. To unmask signals hidden by the reaction solvent, the solvent was removed under reduced pressure. The substitution product is a quaternary amine and is nonvolatile. Product to standard ratios were redetermined in 3/2 (v/v) mixtures of DMSO-$d_6$/$D_2O$, the $D_2O$ serving to exchange obstructing amino group signals. Complete reaction of **2** was observed as evidenced by the disappearance of the methylene signal of **2** at 6.0 ppm. DABCO and **2** reacted quantitatively with a mass balance of 90% referenced to BTAP and 104% referenced to TSP.

Reactions of **1** and **2** with DABCO gave the same substitution product, as evidenced by the same observed chemical shifts in kinetic and mass balance runs (2.5 ($CH_3$), 3.4 (DABCO), 4.7 ($CH_2$), and 8.2 (H-6) ppm) in methanol or in DMSO-$d_6$. The upfield shift of the methylene group is consistent with *N*-alkylation. The possibility of competing formation of the 5-methoxymethyl pyrimidine is unlikely based on its reported chemical shifts, especially for $CH_2$ (4.26 ppm) (*9*). Since excess DABCO was used, the chemical shifts of the substitution product should be that of the pyrimidine-free base because DABCO, being more basic, deprotonates the pyrimidine ring to generate free base.

The substitution product of **1** with aniline was shown to be **3b**. Addition of authentic **3b** to the reaction mixture in DMSO-$d_6$/$D_2O$ confirmed the identity of the product.

The reaction of **1** with 4-aminopyridine gave a product with the same chemical shifts as those for the isolated, authentic derivative.

## REFERENCES

1. WILLIAMS, R. R. (1935) *J. Amer. Chem. Soc.* **57**, 229; WILLIAMS, R. R., WATERMAN, R. E.,

KERESZTESY, J. C., AND BUCHMAN, E. R. (1935) *ibid*, **57**, 536; WILLIAMS, R. R., BUCHMAN, E. R., AND RUEHLE, A. E. (1935) *ibid.*, **57**, 1093.
2. ZOLTEWICZ, J. A., AND KAUFFMAN, G. M. (1977) *J. Amer. Chem. Soc.* **99**, 3134.
3. MATSUKAWA, T., AND YURUGI, S. (1951) *Yakugaku Zasshi* **71**, 1423; (1952) *ibid.* **72**, 33.
4. MURATA, K., HASE, S., IKEHATA, H., AND MITANI, S. (1961) *J. Vitaminol.* **7**, 141; OTAGIRI, Y., UEKAMA, K., AND IKEDA, K. (1975) *Chem. Pharm. Bull.* **23**, 13.
5. ZOLTEWICZ, J. A., AND URAY, G. (1980) *J. Org. Chem.* **45**, 2104; EL HAGE CHAHINE, J. M., AND DUBOIS, J. E. (1983) *J. Amer. Chem. Soc.* **105**, 2335.
6. SHIMAHARA, N., NAKAJIMA, N., AND HIRANO, H. (1974) *Chem. Pharm. Bull.* **22**, 2081.
7. ZOLTEWICZ, J. A., URAY, G., AND KAUFFMAN, G. M. (1981) *J. Amer. Chem. Soc.* **103**, 4900.
8. ZOLTEWICZ, J. A., AND BAUGH, T. D. (1980) *Synthesis*, 217.
9. JORDAN, F., AND MARIAM, J. H. (1978) *J. Amer. Chem. Soc.* **100**, 2534.
10. RISINGER, G. E., BREAUX, E. J., AND HSIEH, H. H. (1968) *Chem. Commun.*, 841.
11. ZIMA, O., AND WILLIAMS, R. R. (1940) *Chem. Ber.* **73**, 941.
12. LIENHARD, G. (1970) *Biochemistry* **9**, 3011.
13. ZOLTEWICZ, J. A., URAY, G., AND KAUFFMAN, G. M. (1980) *J. Amer. Chem. Soc.* **102**, 3653.
14. ZOLTEWICZ, J. A., AND URAY, G. (1981) *J. Amer. Chem. Soc.* **103**, 683.
15. ZOLTEWICZ, J. A., AND URAY, G. (1981) *J. Org. Chem.* **46**, 2398.
16. ZOLTEWICZ, J. A., KAUFFMAN, G. M., AND URAY, G. (1982) *Ann. N. Y. Acad. Sci.* **378**, 7.
17. ZOLTEWICZ, J. A., KAUFFMAN, G. M., AND URAY, G. (1984) *Food Chem.* **15**, 75.
18. ZOLTEWICZ, J. A., URAY, G., BAUGH, T. D., AND SCHULTZ, H. (1985) *Bioorg. Chem.*, **13**, 135.
19. RITCHIE, C. D., AND HEFFLEY, P. D. (1965) *J. Amer. Chem. Soc.* **87**, 5402.
20. LEUNG, C. S., AND GRUNWARD, E. (1970) *J. Phys. Chem.* **74**, 696.
21. BERNASCONI, C. (1973) *in* MTP Int. Rev. Sci., Series 1, Vol. 3, pp. 33–63.
22. BUNNETT, J. F. (1957) *J. Amer. Chem. Soc.* **79**, 5969.
23. AVETA, R., DODDI, G., AND ILLUMINATI, G. (1983) *J. Amer. Chem. Soc.* **105**, 5661; AVETA, R., DODDI, G., ILLUMINATI, G., AND F. STEGEL (1981) *J. Amer. Chem. Soc.* **103**, 6148.
24. OKA, Y., KISHIMOTO, S., AND HIRANO, H. (1970) *Chem. Pharm. Bull.* **18**, 527, 534.
25. MATSUKAWA, T. AND YURUGI, S. (1954) *J. Vitaminol.* **2**, 27.
26 TIMMERMANS, J. (1950) Physico-Chemical Constants of Pure Organic Compounds, p. 303, Elsevier, Amsterdam.